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AN EVALUATION OF THE TESTS BEING USED AT PRESENT TO DETERMINE PREGNANCY

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General acceptance of the various modifications of the Aschheim-Zondek reaction as the method of choice in laboratory diagnosis of pregnancy has occurred during the past seven years in spite of the development of a large number of tests for this purpose. The simplicity of the procedure has permitted experience with large series of cases which in the hands of various workers have yielded a consistent high degree of accuracy not attained with any of the other test methods available. The rapid development in our knowledge of menstrual and reproductive endocrinology has demonstrated that the reaction is dependent upon the production of enormous concentrations of the gonadotropic substance early in pregnancy. This substance which was at first believed to be a pituitary hormone has since been recognized as a placental hormone. That the concentration of this hormone is dependent on the amount of functional decidual tissue present seems to be indicated by its gradual disappearance following fetal death and by the very rapid increase in amounts accompanying the development of hydatid mole and chorion-epithelioma.

Recent outstanding contributions concerning hormone production and relationship during pregnancy substantiate many previous conclusions. Brown and Venning,[1] by frequent quantitative estrogenic and gonadotropic determinations throughout several entire pregnancies, have demonstrated in chart form the enormous concentration of gonadotropic hormone very early in pregnancy, accompanied by low estrin levels. At about 2½ months a sudden decrease in gonadotropic concentration and rise in estrin production reverses

this relationship. Thereafter the gonadotropic concentration remains at comparatively low levels, cyclic estrin fluctuation occurs as in the non-pregnant woman, with the highest concentration being present immediately preceding labor; expulsion of the placenta being followed by rapid disappearance of the high concentration of both substances.

Evans, Kohls, and Wonder,[2] by quantitative determinations of gonadotropic substances in 6 cases of pregnancy, not only confirmed the work of Browne and Venning but established the "peak concentration" of gonadotropic substances at approximately 30 days and the termination of this excessive concentration at about 60 days following the first missed period and in addition made the very important observation that the "peak concentration" of the *urinary* gonadotropic substance was found to be identical with that of the *blood*.

When considered in relation to experience with the various hormone tests for pregnancy diagnosis these observations provide the explanation for the greater accuracy in early pregnancy of those tests which are dependent on the presence of gonadotropic hormone and indicate the reason for the inadequacy of those tests dependent on the presence of estrogenic substances. They also demonstrate the fact that blood serum and urine should be equally effective as media for the tests based on gonadotropic determination and indicate that blood serum might best be used when the test cannot be carried out immediately.

With the preponderance of evidence favoring the modifications of the Aschheim-Zondek reaction, consideration of their respective merits is indicated. The essential difference in the two methods used most frequently is that one utilizes the *mature* female rabbit (Friedman)[3] and in the other the *immature* female (16 to 18 weeks of age) is the animal of choice (Schneider).[4, 5, 6, 7] The adult rabbit, because of greater sensitivity of the ovaries, produces a considerable percentage of incorrect positives in the presence of endocrinopathies or as a result of lack of proper individual isolation. For the same reason, i.e., increased sensitivity, fewer incorrect negatives are encountered. If operation and isolation are alternated the adult animal may be used repeatedly for as many as 6 or 8 tests, while it is advised that the immature rabbit be used for only one test and that autopsy instead of operation be employed. Davy and Sevringhaus,[8] however, report as many as three tests with the same immature animal.

In the author's series of over 1500 cases employing the immature rabbit only one incorrect positive was encountered. (This occurred early in the series and in view of subsequent experience can be at-

tributed only to lack of knowledge of interpretation, the only other errors in the entire series being on the negative side). Confirmation of the lack of incorrect positives with the immature rabbit is contained in the report of Davy and Sevringhaus[8] in a series of 425 cases employing both the Friedman adult rabbit and the Schneider immature rabbit. They obtained a total accuracy of slightly over 90 per cent, stating that while no incorrect positives were encountered with the immature rabbit, 6.59 per cent of incorrect positives were encountered in the series. In contrast, incorrect negatives were encountered more frequently with the immature rabbit, the total incidence for the series being 2.82 per cent.

The Schneider Modification

(1) Immature rabbits—16 to 18 weeks of age (need not be isolated).
(2) Concentrated morning voided specimen of urine.
(3) Filtered immediately preceding injection.
(4) Two injections of 10 c.c. each within 6 to 24 hours into the marginal ear vein.
(5) Autopsy 40 to 48 hours after first injection.

Interpretation:

(1) Flat, opaque, or stellate ovaries with sharp edges and no visible follicles are evidence of excessive immaturity and the test must be repeated.

(2) Rounded ovaries with visible clear follicles and no hemorrhagic or luteinized follicles are interpreted as a negative result if test is done as late as 10 to 14 days following the missed menses and there is no existing endocrinopathy. A negative result obtained at an earlier date or in the presence of previous menstrual irregularity cannot be considered conclusive and necessitates repetition in 7 to 10 days.

(3) The presence of one or more corpora hemorrhagica or corpora lutea denotes a positive result and indicates the presence of decidual tissue which may be normal pregnancy, hydatid mole, chorion-epithelioma, or incomplete abortion—distinction between these factors being dependent on consideration of the history and physical findings in each instance.

(4) Grayish blue opaque appearance of the hemorrhagic follicles, or the reaction described by Rezek[9] may be evidence of fetal death and should be considered as of probable prognostic value.

Limitations of the test

Animal variations and the fact that production of gonadotropic substance probably does not occur in recognizable quantities until 19

to 21 days after conception occurs, will always be responsible for a certain irreducible error regardless of the method employed. Furthermore, since a positive result may occur in hydatid mole, chorion-epithelioma, or extra-uterine pregnancy as well as in normal pregnancy and fetal death, it becomes obvious that interpretation of the tests must in each instance be accompanied by careful consideration of both the history and physical findings. Co-operation between the laboratory technician and the clinician, therefore, becomes an essential factor in attaining the greatest possible accuracy.

Diagnosis of beginning hydatid mole or chorion-epithelioma has been suggested by dilution methods based on the enormous concentration of gonadotropic substance which results from the increased growth of decidual tissue. Great care must be exercised in arriving at diagnosis in this manner as demonstrated by the rather frequent reports of the evacuation or removal of normally pregnant uteri. This indicates that even in certain cases of normal pregnancy the concentration of gonadotropic substances may be almost as great as in pathologic conditions. Further quantitative investigations along these lines will undoubtedly improve the diagnostic scope of the test. As a prognostic measure following the removal of hydatid mole or chorion-epithelioma the test is invaluable and routine repetition at frequent intervals is indicated in every case of this type.

Diagnostic results in extra-uterine pregnancy have been notably unsuccessful with either of these methods and the many reports of greater accuracy in the diagnosis of this condition by estrogenic methods merit further consideration. The probable explanation may be that due to imperfect placentation insufficient amounts of gonadotropic substance are produced with the likelihood that the ovary may under these circumstances produce sufficiently large amounts of estrin to permit earlier recognition of its presence. The numerous conditions other than pregnancy which may give rise to excessive amounts of estrin, combined with the technical difficulties of the procedure, have prevented its more general application. Here again further quantitative studies should be of value. The estrogenic methods most frequently employed have been those of Mazer and Hoffman,[11] and the Bitterling test of Kantor, Bauer, and Klawans.[12] Recent investigation (Kleiner, Weisman and Mishkind[13]) has revealed the specificity of male hormone in producing lengthening of the ovipositor of the Bitterling, thereby removing the Bitterling test as a diagnostic measure for pregnancy determination.

In a previous publication[5] the observation was made that a "fading reaction" accompanied fetal death and incomplete evacuation of the uterus and persisted only as long as *functional* decidual

tissue remained in the uterine cavity, completely negative results being observed upon cessation of function even though the tissue be retained in the uterus. Rezek,[9] in a recent publication, using the immature rabbit, reported a reaction characterized by peripheral hemorrhage in large otherwise clear follicles. Accuracy of 95.3 per cent was obtained in a series of 86 patients in the determination of fetal death. In late pregnancy Rezek finds the reaction within 24 hours following cessation of the fetal heart tones. In this instance also investigations of quantitative hormone production may be of significance in providing an explanation of the results obtained.

In two small series of cases at the Illinois Research Hospital, Rezek[10] has reported the following results:

Visscher Test—64 cases.			
17 males	7 positive	10 negative	
27 pregnancies	17 positive	10 negative	
20 non-pregnant	7 positive	13 negative	
Antuitrin S Skin Test—100 cases.			
15 males	5 positive	10 negative	
57 pregnancies	37 positive	10 negative	10 ?
28 non-pregnant	10 positive	14 negative	4 ?

Conclusions

(1) At the present time evidence indicates that the modifications of the original Aschheim-Zondek test give the greatest accuracy of results in the diagnosis of early pregnancy, and that they may be used as prognostic measures in hydatid mole, chorionepithelioma, incomplete abortion, and fetal death.

(2) Recognition of the limitations of the test and the advantages and disadvantages of the various test animals, in addition to careful consideration of clinical history and physical findings should be conducive to increased accuracy.

(3) Further investigation of quantitative hormone production and relationship during pregnancy may be expected to extend the scope of these tests.

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SOME LABORATORY FINDINGS IN SICKLE-CELL ANEMIA*

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The disease of sickle-cell anemia is not a rare one, but it is only of recent years that interest has been stimulated in the subject, as shown by the increased number of articles appearing in print. Herrick[1] reported the first case in 1910, and since that time much work has been done to try to determine the cause as well as the cure for this specific disease. Although some progress has been made, the results thus far are somewhat indefinite due to the varying aspects of the disease and to the short duration of the experimental work.

The majority of the cases appearing in literature are found in the negro race. While the disease occurs mostly in children, the average age is thirteen years. It predominates in males. The youngest case recorded is one of seven months and the oldest is one of a person sixty-two years old. A decreased resistance is brought about by this condition, thus making way for various diseases which cause death. Few patients live beyond thirty-five years. The pathological changes are limited apparently to the spleen, liver and bone marrow. In persons over sixteen years of age ulcers are apt to occur. Below this age ulceration occurs rarely, having been reported in but two cases[2].

There seems to be a difference of opinion concerning the diagnosis of sickle-cell anemia. Some think that a tentative diagnosis can be made upon the clinical picture alone, while others think the diagnosis is hidden until revealed in laboratory findings[3]. There are two phases of this disease: (A) Active, in which the sickling of the red blood cells occurs in the circulating blood and anemia develops. (B) Latent, in which the patient shows only the sickle cell trait. This last condition is spoken of as sicklemia. Here the red blood cells undergo transformation into sickle shapes in anaerobic preparations while the patient may not show any signs of circulatory sickling nor of anemia. The cause of the sickling is not known but the anemia itself is due to hemolysis and phagocytosis of the red blood cells[4]. In our study of both the stained cells and wet prep-

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aration we have been unable to find phagocytosed red blood cells. In the active phase there may be long periods when no signs or symptoms of the disease are present. The sickling trait, however, persists throughout life.[5] The tendency to sickle-cell formation is considered to rest on a hereditary predisposition and is a familial trait.

One of the essential features for diagnosis is obtained by means of the anaerobic preparation. This is done by placing a drop of blood upon a slide, placing a cover slip over the drop and sealing with petrolatum or paraffin. The slide is then placed in the incubator at 37.5° for 24 hours and observed at intervals to determine the rate of sickling. Temperature effects the sickling; heat tends to hasten it while cold retards it. In leaving preparations at room temperature we found the sickled cells became round in about four weeks. Quite a few workers have done routine examinations on negroes and found the percentage having sicklemia to be variable up to 7.5%[6]. In a series of a hundred negroes which we have examined by the wet preparation method, we have found sickling in 7% of the bloods examined. In one instance we examined the bloods of a new born child and its mother. The mother's blood showed sickling but the child's blood did not. The mother complained of having been sick all her life. The mother's mother was then examined and her cells were strongly sickled but she insisted that she had always been well. In another instance we found sickling in a negro diabetic about sixty years of age.

Diggs[7] believes that the rate of sickling is related, not necessarily to the immediate severity of the anemia, but to its chronicity. Patients who have no sickle cells in the circulating blood and show a slow rise in percentage of sickled forms, present a better prognosis than the ones with a quick rise and a high percentage of sickling. These observations are, however, based upon a limited amount of evidence. The aforementioned author finds the rate of sickling extremely variable, depending upon many factors such as: method of collecting, exposure to air, size of drop, character of spread, weight of cover glass, hydrogen ion concentration, bacterial growth, minor variations in temperature, exposure to light, and effectiveness of seal. Several preparations on different days should be made.

Hahn and Gillespei[8] found that the lower the pH below pH 7.4 the better the sickle cells form in the presence of carbon dioxide. With oxygen the sickle shape is reversed to discoid. The lowest oxygen concentration bringing about reversal to discoid is six per cent. It is their opinion that cells are produced in the blood by conditions of anoxemia.

An interesting theory is advanced by Robinson[9]. A study of the anatomy of sickle-cell anemia reveals an over activity of the cells of the reticulo-endothelial system. Since this system plays a big part in pigment metabolism, it makes it reasonable to believe that if any pathological change did occur in this system, it would be in the individuals where there is a demand for increased pigment metabolism, such as in the negro or in races with moderate skin pigment.

Several cases of sickle-cell anemia have been reported as occurring in the white race with reasonable assurance of there having been no admixture of negro blood. Perhaps the most interesting of those appearing in literature is reported by Cooke and Mack of St. Louis, Missouri[10]. The two youngest children of a family of six, age one and three, respectively, showed typical sickling and the father's blood showed sickling in the wet preparation after from twelve to twenty-four hours.

Diggs, Pulliam and King[11] report a very complete study of the bone marrow and the roentgenographic appearance of the bones in sickle-cell anemia. Microscopically they found bone marrow presenting a picture of great cellularity and congestion with many sickled erythrocytes in the capillary and tissue spaces. The mononuclears contained ingested red blood cells. They found the bone changes to be non-specific. The degree of bone changes appeared to have no relationship to the severity of the anemia. The majority of the patients with sickle-cell anemia examined revealed no abnormality in size shape or density of the bones.

No specific treatment for this disease seems to have been established as yet. Transfusions give only temporary relief. Liver therapy fails to help the patient. Wholesome food, plenty of rest, fresh air and avoidance of infections are advocated[12]. Cases are reported in which splenectomy has benefited the patients. Although the blood counts improve, the anemia and sickling persists. The patients seem more comfortable and less susceptible to the usual complications which characterize the disease[13].

In our work with patients having sickle-cell anemia, our laboratory findings are not strikingly different from most of the other cases reported. There seems to be a greater variation in the number of nucleated red cells and eosinophils in the blood counts done over a period of time, while the total red and white count, the hemoglobin and the reticulocyte count are more constant.

The most interesting incident of this disease to appear in our number of cases is that of a negro family in which two children have active cases of sickle-cell anemia. This family seems to be

far above the average in intelligence and has been very cooperative, thus enabling us to study the entire family.

A brief report of the laboratory findings on these cases follow: A boy, age seven, entered our clinic complaining of kidney trouble. The laboratory report was quite typical of sickle-cell anemia. Hemoglobin 50%, Sahli, color index 1.2, red count 2,130,000, white count 12,400. The differential leukocyte count showed nine eosinophils and a non-filament index of 12. The red cells showed marked achromia, some anisocytosis, poikilocytosis and polychromatophilia and eight normablasts with typical sickling of many cells. The cells in a wet preparation were sickled almost to a hundred per cent. Reticulocyte count 8.2%. Sedimentation rate was normal. Fragility test—hemolysis began at .36 and was not complete at .12. Coagulation two minutes. The most marked findings in the urine were that urobilinogen and urobilin were strongly positive.

A few months later this boy's sister, age thirteen, entered the City Hospital for a tonsillectomy. She had suffered from frequent sore throats for five previous years. Again, the laboratory report showed a typical picture of sickle-cell anemia. Hemoglobin 50%, Sahli, color index 1.1, total red count 2,350,000, total white count 15,000. The differential count showed two eosinophils and a non-filament count of 34. There was some sickling of the red cells on the stained preparation with two normablasts. The reticulocyte count was 20.6%. The sedimentation rate was normal, the fragility test—hemolysis began at .30 and was not complete at .12. In the wet preparation, practically all cells were sickled. The icteric index was 30, the Vanden Bergh- delayed direct, positive and indirect one milligram. Urobilinogen and urobilin were strongly positive in the urine.

The other members of this family, the mother and a twin sister to the boy, age seven, were then examined. The twin sister did not show such typical signs of this anemia. No sickle cells were present on the stained slide but at least 95% of the cells sickled in the wet preparation after a 24 hour incubation. The hemoglobin was 75%, color index .9, total red count 4,260,000, total white 11,900. Seven eosinophils were noted on the differential leukocyte count. A trace of urobilinogen was present in the urine, while the urobilin was negative.

A routine examination of the mother's blood showed nothing abnormal. After incubation, the wet preparation contained only a few cells which were definitely sickled. A trace of urobilinogen was found in the urine.

From the foregoing facts established by various investigators and observed by ourselves, it becomes apparent that aside from the sickling of the erythrocytes, there are no remarkable or outstanding laboratory findings in sickle-cell anemia. The importance of anaerobic wet preparations as a means of diagnosis cannot be over emphasized. If this were adopted as a routine measure in examination of negroes' blood, we would no doubt be able to discover more cases of sickle-cell anemia than has been done in the past.

Prepared under supervision of G. W. Kittelberger, M.D., Assistant Professor of Bacteriology and Clinical Pathology.

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DIFFERENTIAL DIAGNOSIS OF MONONUCLEAR CELLS IN BLOOD

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The study of a stained slide in the differential count of a blood smear offers possibilities and stimulates interest in one phase of laboratory work that has no equal. To find the types of leucocytes which present themselves in variations of pattern, coloring and number makes for an ever-increasing enthusiasm.

It is the purpose of this discussion to deal with the "mononuclear cells" of the blood, and to present a picture of the monocyte which perhaps is the most variable form of leucocyte.

The monocyte is well known as to morphology, but its origin and relationships to other cells of the circulating blood and tissues have provided controversy. Variations in the methods used in hematology and histology, and differences in interpretation and identification may explain the fact that the monocyte has been an oft discussed cell, especially as to origin.

The monocyte was first recognized in the dry smear. Ehrlich and Einhorn have described two groups of large mononuclear cells; the large mononuclear leucocytes with round or oval nuclei, and "transitional" cells with notched, kidney-shaped or polymorphous nuclei. The latter were believed to be intermediate forms between large mononuclear and polymorphonuclear leucocytes. Staining methods changed this theory, and from the studies of Pappenheim and later Pappenheim and Ferrata the term "large mononuclear leucocyte" came to designate the group, with both forms included. The Ehrlich classification included with what we now call monocytes, the large lymphocytes. These latter cells were placed in the class which includes small and medium-sized lymphocytes, while "monocyte" (Pappenheim and Ferrata, 1910) refers to the large mononuclear cell with phagocytic behavior.

The classification of the mononuclear leucocytes includes a survey of the characteristic forms. The three forms are the lymphocytes, mature and immature, and monocyte, also known as endothelial leucocyte, or endotheliocyte.

The mature lymphocytes are small mononuclear cells without cytoplasmic granules. Their size is about that of an erythrocyte, or slightly larger, 6-10 microns. The single, round, or some-

times indented, nucleus is sharply defined with heavy blocks of chromatin staining blue with the Wright's stain, and parachromatin staining pink. There is a gradual transition between the chromatin and parachromatin, a blending of the two. The narrow zone of cytoplasm stains a "robin's-egg blue."

The larger cell of this group, 12-15 microns, has a paler nucleus with a clear perinuclear area, and more abundant cytoplasm. Often this cell is confused with the monocyte. It is designated as an immature lymphocyte which becomes smaller as it matures. The nuclear pattern is looser than that of the mature form, thereby making it appear paler. The cytoplasm may show a few (5-10) azurophilic granules, rounded and discrete. These reddish-purple granules are larger than those of the polymorphonuclear leucocyte. With the mature form, this type comprises about 25-35% of the leucocytes and originates in lymphoid tissue, including that of the bone marrow.

The "monocytes," "endothelial leucocytes," or "endotheliocytes" make up 2-6% of the normal differential count and are the largest cells of the circulating blood. They are two to three times the size of the erythrocyte, measuring 14-20 microns. The single nucleus is lobulated, deeply indented, horse-shoe shaped, or less often, round or oval, and is placed eccentrically. A wide slate-colored or "muddy" blue zone of protoplasm is frequently filled with reddish granules, the so-called azure "dust," less distinct than the granules of neutrophilic leucocytes. The nuclear chromatin is in strands and linear arrangement, with wide spaces of parachromatin in sharp distinction, giving the effect of a less densely stained nucleus. There is no perinuclear clear area.

The origin of this cell is little known and much study has been done to place it definitely. Beginning with Ehrlich, the leucocytes have been classified as belonging to the myelogenous and lymphatic systems:—the granulocytes developing from the myelogenous system, (the marrow of the short bones and pathologically, the long tubular bones and intramedullary centers), and the lymphocytes from the lymphatic system (spleen, lymphatic glands, lymphatic tissue). There were also monocytes in a rather doubtful position, but now considered by some as representing a third system.

On this point, hematologists have divided into three groups of thought. Representing the idea that there are three systems of hematopoietic organs, Schilling and others hold that the monocyte is independent of the others, coming from a general system, the reticulo-endothelium of spleen and liver, bone marrow and connective tissue. This view described as "trialistic," is opposed to the

"unitarian" view of Weidenreich, Maximov, and others, that all blood cells are derived from the lymphocytes or lymphoid, primary forms; and to the "dualistic" theory of Ehrlich, Naegeli, Tuerk, who believe that the myelogenous and lymphatic cells represent the primary forms, not differing morphologically, but according to arrangement in tissue, and capacity for development. The "trialistic" theory explains the independent position of the monocyte. Among many authors, Cunningham, Doan and Sabin whose work with supra-vital staining is well known, have accepted it.

The study of the monocyte shows that the cell presents great variation in structure, and its diagnosis as a large mononuclear leucocyte is based on the composite picture. The size of the cell may be sufficient, but the fact that immature lymphocytes may be as large, complicates matters. The azure "dust" granules as usually present can offset the question of size, but in their absence within the normal other characteristics must be used. In the nucleus the chromatin may vary from delicate, light staining network to deep staining coarse strands. There may not be a sharp delineation between chromatin and parachromatin, for the wide spaces of parachromatin, by a reduction in amount, will tend to make a blending of the two, as in the immature lymphocyte. When these are not definite enough, the color of the cytoplasm and the fine dust-like azure granules show the monocytic type. Sometimes the slate-blue cytoplasm is replaced by a pale, clear blue, and then the nuclear pattern is constant. At least one characteristic is there, either slate-gray blue cytoplasm, azure "dust" granules, nucleus with sharp distinction of chromatin and parachromatin, in a large mononuclear cell. If the normal includes all these, the diagnosis is simple. Schilling says that "those cells alone should be considered monocytes which have absolute resemblance to normal types; all doubtful elements should be considered lymphocytes; and in leucemia, as belonging to the stem or parent cell."

In the study of the pathological monocyte, reference will be made to types in two groups of diseases: (1) Severe infection, acute and chronic infections (measles, scarlet-fever, influenza, variola, pneumonia, tuberculosis, syphilis, etc.) malignant tumors, Hodgkin's disease, Banti's disease, malaria, pernicious anemia, anemia pseudoleucemica infantum (von Jacksh's); and (2) monocytic leucemia, leucemic reticuloendotheliosis, and subacute bacterial endocarditis.

In general the size of the cell varies greatly, often smaller than normal, but larger during regeneration with giant monocytes often three times normal size in pernicious anemia. The nuclear shape is usually within normal limits, but in pernicious anemia and mono-

cytic leucemia there may be extreme lobulation and bizarre forms in immature and mature monocytes. Alder (1922), and Naegeli (1923) describe monocytes with segmented nuclei in pernicious anemia, the mycloses, and secondary anemia. This is regarded as an indication of regenerative activity of the bone marrow.

The pattern of chromatin and parachromatin in pernicious anemia shows the delicate, fine framework, within normal range, but of the more delicate type of the normal. Some authors claim presence of nucleoli in nuclei, but others report none. The coarse-nuclear pattern, an opposite extreme of the normal range, occurs in infectious and chemical toxic conditions. The chromatin is in coarse strands and heavy masses, irregular throughout; and the separation from parachromatin is very distinct.

In monocytic leucemia, the immature monocytes are traced back to the myeloblasts, which according to Naegeli place this disease as a temporary variation of acute myelogenous leucemia, not a separate form. The cell nucleus here is of myeloblastic type: there is a thin nuclear membrane and diffuse, uniform distribution of chromatin, a nucleolus with chromatin condensed about it. This chromatin is in the form of fine dust-like particles merging into delicate strands over a part of the nucleus, and described as dense stippling combined with finely reticulated meshwork. Sometimes a few strands are slightly coarser, and the cell is somewhat beyond a typical myeloblast.

The cytoplasm in monocytes of monocytic leucemia varies from the deep blue of myeloblasts to light slate-gray of mature monocytes. An intense muddy-blue or dark-gray cytoplasm, with mature nucleus comes in acute, infectious diseases, malaria, anemia pseudoleucemica infantum. A reddish cast may be observed in the cytoplasm in carcinoma.

The azure granules of monocytes may be very few in number or entirely absent in pernicious anemia, secondary anemias, or myeloses. Some authorities claim a "granule-poor condition" to be more common in pernicious anemia than in secondary anemias. On the other hand a majority of cells contain abundant "azure dust." In leucemic reticuloendotheliosis and subacute bacterial endocarditis, the lessened number of azure granules is quite pronounced. In the monocytic leucemia (Naegeli type) the state of the azure granules is quite variable. A typical myeloblast contains granules of its own type. In a pathological monocyte the monocytic granules appear early, and as differentiation proceeds increase in number and in size. Then reduction occurs, and there develops the azure "dust" of mature monocytes.

In severe infections and toxic conditions there are monocytes abundant with coarse azure granules, as round coarse particles staining deeply with the basophilic part of Romanowsky dyes. The monocytic cytoplasm is muddy blue, and the nucleus is coarse.

In the pathological monocytes of pernicious anemia, and monocytic leucemia, vacuoles occur in isolated cells. These vacuoles are common in subacute bacterial endocarditis, and contain cellular debris in varying stages of digestion.

Naegeli reports pigment granules in monocytes in malaria. Phagocytosis of erythrocytes by monocytes occurs in such conditions as severe pernicious anemia, severe secondary anemia of newborn, myelogenous and monocytic leucemia, tuberculosis, sepsis, and subacute bacterial endocarditis. Ingestion of polymorphonuclear neutrophiles is found in monocytic leucemia.

It is evident from these observations that the pathological monocytes present a most varied and colorful picture. It is evident also that they do not occur to a great extent in the general routine of blood studies. These facts perhaps account for the fact that the monocyte is considered a most difficult cell to place as to origin and relationship. Even in its normal phase, the variations in nuclear structure, arrangement of chromatin and parachromatin, the color changes of the cytoplasm, and presence and abundance of azure granules furnish valuable studies. It is enough to call to mind the normal picture of the mature monocyte: the large mononuclear cell with its delicate nuclear structure, where chromatin and parachromatin stand out distinctly; the abundant slate-gray cytoplasm with azure "dust" granules; the absence of a perinuclear, clear area; and the eccentric, odd-shaped nucleus. When cells of this type are seen, whether the origin be from lymphocytes, myelocytes, or general independent form, there is before you the mononuclear cell of mysterious ancestry and picturesque beauty.

THE REMOVAL OF WRINKLES FROM PARAFFIN SECTIONS

By LOUIS C. L'ARCHEVEQUE, M.T.

Compared with the vast amount of work done in other medical fields, little or nothing has been said pertaining to paraffin sections. The purpose of this publication is to bring to fellow technologists a more simple and less tedious method of removing wrinkles from sections to be stained by the paraffin procedure.

It is the usual custom in the paraffin method to place your sectional strips cut from the paraffin block in ice-water, which serves the purpose of hardening them, and also aids in the arduous task of separating one section from another.

The general procedure from there is the application of the section to a glass slide covered with a fixing reagent.

The next step is the placing of the slide with the section, into a container of hot water, this helps to eliminate some of the wrinkles caused by the extreme coldness of the water.

However, this is not always the case, because it is not always possible to get a section mounted on the glass slide completely void of all wrinkles.

The following method has been tried and proven from an economical and practical standpoint to be most efficient:

After experimenting with various percentages of alcohol, it was found that 60% alcohol was sufficient to prevent any further destruction of the prescribed section.

The complete dehydrating of the section is the important factor in the elimination of wrinkles.

This wrinkle removal process is brought about by the pseudo-ameboid movement assumed by the section because of the rapid absorption of the water.

It produces a quick circular motion on the surface of the water which in turn spreads the section out in its entirety.

Procedure

Place your sectional strips into a shallow dish containing 60%

alcohol instead of ice water for about 15 to 20 seconds before mounting, to ensure partial dehydration.

Remove one section at a time to your glass slide and place on the surface of the warm water which is kept between 45 and 50 degrees centrigrade.

Theory

The moment the section contacts the water, absorption begins immediately causing two distinct reactions:

(a) pseudo-ameboid movement,
(b) expansion of the section due to absorption.

The result of those reactions eliminates any overlaping of the section. You may find in using this method upon different specimens brought up to the laboratory and sent through this procedure, that some sections will segregate themselves immediately upon uniting with the 60% alcohol, others do not.

Upon application of a few stabs with a pointed instrument on the connecting line of each section you will find comparatively little resistence before the section has divided.

EDITORIAL

INCREASING MEMBERSHIP

In the preceding issue of the Journal, it was pointed out that much depends upon the membership body of the American Society of Medical Technologists as well as upon its officers. The immediate contacts made by the officers would obviously be smaller, even though this may be supplemented in part by the yearly issue of the Journal mailed to all Registered Technologists.

We here again urge that a continuous campaign be conducted with the thought in mind to contact prospective members through the entire membership body.

Beyond a doubt it would be well for all Medical Technologists to become members of the American Society of Medical Technology. There is no other national society wherein only Registered Medical Technologists may hold membership and it is the only society wherein the code of ethics formulated by the Board of Registry is closely adhered to.

As membership in the American Society of Medical Technology increases, there is no doubt that a larger Journal will be published containing more articles of interest to all, since the number of articles received now is greater than the available space.

By personal contact more results for increasing membership will be achieved. This has been proven to be a fact in many similar instances. Now—the foundation is layed, the material assembled; fellow-member-workers—let us build!

ABSTRACTS

A NEW TITRATION METHOD FOR SUGAR DETERMINATION: Nobuzoo Fujii and Nankoo Akutsu. Jour. Biol. Chem., Vol. 25, No. 2, March, 1937, p. 237.

The authors describe a titration method that they say avoids the errors of the older methods, is easier to carry out and is cheaper.

CELL SIZE AND METABOLIC ACTIVITY AT VARIOUS PHASES OF THE BACTERIAL CULTURE CYCLE: Evelyn Huntington and E. A. Winslow. Jour. of Bact., Feb., 1937, p. 123.

Cultures go through a definite cycle with metabolic activity increasing first, cell volume increasing next and ultimately an increase in cell division rate.

INVESTIGATION OF FUNGOUS FLORA OF APPARENTLY NORMAL SKINS, J. G. Downing, R. N. Nye and S. M. Cousins. Archives of Dermatology and Syphilology. 35:1087, June, 1937.

Three hundred scrapings from apparently normal skins of 100 hospital patients revealed pathogenic and saprophytic fungi and various organisms including staphylococcus albus and citreous, streptococcus viridous, diphtheroids and others. Staphylococcus aureus and beta hemolytic streptococci were never recovered.

RELATION OF FUNGUS INFECTION OF GRAIN CROPS TO VASOMOTOR DISTURBANCES IN MAN: J. E. Klein, Annals of Internal Medicine, 10:1708, May, 1937.

Certain vasometer disturbances such as Buerger's disease, Raynaud's disease, acrodynia, and acroparesthesia may be due to chronic intoxication as a result of ingestion of foods subject to fungus infection over a period of years. Experiments with small doses of ergotamine tartrate over a period of two months caused marked constriction in arteries of tails of rats on microscopic examination. The importance of studying plant pathology in relation to human health is emphasized.

THE RELATION OF PROTEIN TO HEMOGLOBIN BUILDING: P. B. Pearson, C. A. Elvehjem and E. B. Hart. Jour. Biol. Chem., Vol. 119, No. 2, p. 749, July, 1937.

The experiments showed that rats on diets with protein rations either qualitatively or quantitatively inadequate for growth were less able to replace hemoglobin than those on a more adequate diet. If sufficient iron and copper were fed, the animals sometimes maintained a normal hemoglobin but apparently at the expense of their own body protein.

BOOK REVIEWS

CLINICAL URINALYSIS AND ITS INTERPRETATION by Robert A. Kilduffe, A.M., M.D., F.A.S..C.P., Director of Laboratories, Atlantic City Hospital; Pathologist, Atlantic County Hospital for Tuberculous Diseases; Serologist, Atlantic County Hospital for Mental Diseases; City Bacteriologist, City of Atlantic City; Serologist, Municipal Hospital for Contagious Diseases, Atlantic City; Pathologist, Betty Bacharach Home for Crippled Children, Longport; Pathologist, Jewish Seaside Home, Atlantic City; Formerly Major, M.C., U.S.A. Published by F. A. Davis Company, Philadelphia. Price $4.00.

This volume of 428 pages with 40 illustrations gives the most practical, accurate and sufficiently delicate methods of urinalysis for clinical purposes. The methods described are complete in technical detail and, as usual two or more methods are described for the same test, being given in the order of preference. The author is to be commended for his thorough treatment of the subject of urinalysis and particularly for the clinical interpretation and significance of all findings. The book is intended for clinicians rather than technicians. Although the technic is given accurately and in detail the clinical interpretation is the more outstanding feature. Chapters are devoted to the examination of urine for life insurance, the equipment of the office laboratory for urinalysis, formulae for reagents and test solutions and miscellaneous tables.

ATLAS OF HEMATOLOGY, by Edwin E. Osgood, M.A., M.D., Assistant Professor of Medicine and Head of Experimental Medicine, and Clarice M. Ashworth, Medical Illustrator, both of University of Oregon Medical School, Portland, Oregon. J. W. Stacey, Inc., Publishers, 236-238 Flood Building, San Francisco, California, 1937. Pp. 255 and 325, illustrations and frontispiece in color. Twenty-two tables. Price $10.00.

The title for this work is well chosen and the arrangement of the material is excellent. The most outstanding feature is the colored plates which are exceptionally well done and are as true to the actual colors of stained specimens as it is possible to get them. Each cell or group is numbered and then in the text is named and described under the same number. The illustrations are large and in some instances a whole page is used for one colored plate of a cell. Every cell, normal and abnormal, of both the erythrocyte and leucocyte series, which a hematologist will encounter in his work, is illustrated in full color, numbered and described in detail.

Chapter I gives the general principles of hematologic diagnosis with subtitles for the way to use the Atlas, examination of patients with hematologic disorders, nomenclature, cell identification and histogenesis of blood cells. The author states that the section on cell identification is the most important part of the book. A system of tables has been developed and explained by the author which should "permit any one to identify any cell encountered in a properly stained preparation."

Chapters II-X include the 325 colored illustrations with a description in detail of the cells of the blood and sternal marrow, size of the cells in micra, the source from which obtained and any deviations from the typical cell type. The lymphocyte, monocyte, granulocyte, plasmacyte and erythrocyte series are thus described and illustrated.

Chapters XI-XVII discuss the general principles of diagnosis of hematologic disorders, presents tables of differential diagnosis and a concise description of the characteristic hematology of each disease. There are twenty pages of references arranged under the chapter headings to which they pertain in the Atlas.

To those doing any sort of work in hematology the Atlas of Hematology cannot be recommended too highly, as the book would be found to be indispensable once it were placed at their disposal.

NEWS AND ANNOUNCEMENTS

REGISTRY OF MEDICAL TECHNOLOGISTS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

As of January 1, 1938, the new educational requirements for admission to an approved training school for Medical Technologists go into force. A schedule of the chemistry, physics and biology credits follows:

TWO YEAR COLLEGE COURSE FOR ADMISSION TO APPROVED TRAINING SCHOOLS FOR MEDICAL TECHNOLOGISTS

Biology: 15 quarter hours (10 semester hours) of which at least 9 quarter hours (6 semester hours) must be Zoology.

Bacteriology: At least 5 quarter hours or 3 semester hours. (If not available, other branches of Biology may be substituted).

Chemistry: General Inorganic Chemistry. At least 12 quarter hours (8 semester hours), including 6 quarter hours (4 semester hours) in laboratory.

Organic Chemistry. A complete course, at least 9 quarter hours (6 semester hours), with not less than 2 quarter hours of laboratory.

Quantative Analysis. 5 quarter hours or 3 semester hours.

Physics: At least 12 quarter hours (8 semester hours), with 3 quarter hours (2 semester hours) of laboratory. Either High School or College Trigonometry prerequisites to Physics.

English: 9 quarter hours (6 semester hours) in English Composition and Rhetoric.

Electives: Sufficient to give a total of 90 quarter hours (60 semester hours) of college credit. Sociology and Psychology are recommended for electives.

NATIONAL

Minnesota

With Mr. C. H. Winbigler as president of the newly organized Minnesota Society of Medical Technologists, the Membership Committee under the chairmanship of Sr. M. Edeltrude, C.S.J., undertook a membership drive. To accomplish this a questionnaire was prepared and mailed out to two hundred non-registered members who are employed in the state. This questionnaire solicited the necessary information to give each individual his proper status relative to applying to the central office in Denver for registration. The result was that forty-five applications for registration came through the president's office and were eventually forwarded to Mrs. Anna R. Scott for disposition. Other applications were mailed directly to the Denver office. The possibility of a great number of Minnesota Medical Technologists becoming registered this year seems promising.

At the time the non-registered circulars were mailed out, an appeal was sent to about 150 registered members of the state encouraging them to become members of the new state society. Until definite specifications to the contrary, membership is obtained by paying the membership fee of $2.00, and showing credentials that the individual is nationally registered.

Mississippi

Mississippi Valley Medical Society Award—The Mississippi Valley Medical Society offers a cash prize of $100.00, a gold medal and a certificate of award for the best unpublished essay on a subject of interest and practical value to the general practitioner of medicine. Entrants must be ethical licensed physicians, residents of the United States and graduates of approved medical schools. The winner will be invited to present his contribution before the next annual meeting of the Mississippi Valley Medical Society (September 28, 29, 30, 1938), the Society reserving the exclusive right to first publish the essay in its official publication—the Radiologic Review and Mississippi Valley Medical Journal. All contributions shall not exceed 5,000 words, be typewritten in English in manuscript form, submitted in five copies, and must be received not later than May 15, 1938. Further details may be secured from Harold Swanberg, M.D., Secretary, Mississippi Valley Medical Society, 209-224 W. C. U. Building, Quincy, Ill.

Texas

The fifth annual convention of the Texas Society of Medical

Technologists was held in Fort Worth at the Texas Hotel, October 8th and 9th, with Ida F. Levinson, the President, presiding. The meeting was attended by technologists from four states, Texas, Oklahoma, Arkansas and Louisiana, and an excellent group of scientific and commercial exhibits were presented.

The invocation was given by Dr. Everett B. King, and the address of welcome by Dr. R. B. Anderson, assistant editor of the Texas State Journal of Medicine. The morning of October 8th was devoted to the transaction of business. At noon a joint luncheon with the Fort Worth Rotary Club was held.

The afternoon session, with Nan Buffington presiding, was devoted to the presentation of the following papers:

"Examination of Cerebro-Spinal Fluid Contaminated With Blood," V. G. Isvekov, M.D., Houston, Texas.

"Laboratory Diagnosis of Malta Fever," Blanche Greenlea, M.T., Fort Worth, Texas.

"Venereal Diseases," I. P. Barrett, M.D., Fort Worth, Texas.

"The Value of Organization to Medical Technologists," H. A. Bardwell, M.T., San Antonio, Texas.

"The Need For Standardization of Hemaglobinometers," J. E. Storey, M.T., Abilene, Texas.

The scientific session was followed by round table discussion. At 8:30 p. m. a banquet and dance was held, with Mr. Bardwell presiding, and Drs. Terrell, Rodgers, Owens and Isvekov as special guests.

October 9th the morning session, at which Marian A. Baker presided, was devoted to the following papers:

"Autogenous Vaccines," Clyde C. Hayes, M.T., Waco, Texas.

"Etiology and Laboratory Findings In Poliomyelitis," Ann Snow, M.T., Little Rock, Ark.

"Blood Grouping," William Rosenblatt, M.D., Wichita Falls, Texas.

"Bacteriological Diagnostic Methods in Clinical Practice," Lucille Brown, M.T., Oklahoma City, Okla.

"Acidosis," O. T. Woods, M.D., Dallas, Texas.
A round table discussion followed.

A luncheon at the Fort Worth Club was held at noon, at which

Dr. Stout presided; Dr. Hulsey discussed the problems of technologists and Dr. Hood talked on "Purpura And Its Laboratory Findings."

That afternoon a motion picture entitled "Hospital Conditions In India" was presented by William Webb, M.D., Fort Worth, Texas, and the following papers read:

"Blood Glucose Determination In Hypoglycemic States," Marian A. Baker, M.T., Wichita Falls, Texas.

"Intestinal Parasites," H. E. Wright, M.D., Dallas, Texas.

A business session and election of officers followed, during which Waco was selected as the convention city for 1938, and the following officers elected:

President—Marian A. Baker, M.T., Wichita Falls.
President-Elect—J. E. Storey, M.T., Abilene.
Vice-President—Clyde C. Hayes, M.T., Waco.
Secretary—Nylah Tom, M.T., Austin.
Treasurer—Rose Matthaei, M.T., Palestine.

Executive Committee

Tennie J. Bryson, M.T., Wichita Falls.
Anna Lou Smith, M.T., Fort Worth.
Dorothy Hall, M.T., Dallas.
Belle Bowers, M.T., Santa Anna.
Sr. M. Monica Grant, M.T., San Antonio.
Geo. T. Thomas, M.T., Beaumont.